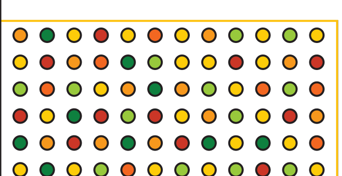
OneArray™

Human & Mouse
DNA Microarray

User Guide



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Notice to the User

It is important that users read the entire manual before commencing work.

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User Guide and Technical Support

Electronic version of this manual is available on the enclosed Product Support CD, and online at:
www.phalanxbiotech.com

To reach technical support by telephone, call
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Feedback

We welcome your feedback regarding our products and this manual. Please contact us at:
feedback@phalanxbiotech.com

All comments are welcome.

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Last updated July 2008

Thank You

Phalanx Biotech Group would like to extend special thanks to our customers who have provided feedback that enabled us to improve the OneArray User Guide.

Table of Contents

Getting Started

Please read the introductory information below to help familiarize yourself with OneArray™ before use.

Product Contents

- Human or Mouse OneArray™ DNA Microarray
- OneArray™ Hybridization Buffer Tube
  - Each tube contains buffers sufficient for 5 to 10 microarray hybridization procedures
- Spare round cap tube
- OneArray™ User Guide
- Spotted Region Guide
- Product Support CD, which contains the following:
  - Sample Images
  - OneArray .gal file
  - OneArray gene list and probe sequences
  - OneArray microarray layout
  - OneArray Control Probe list
  - OneArray User Guide (electronic version)
  - SimpleMeasure experimental control analysis program

Other Necessary Apparatus (Not Supplied)

Apparatus

- Water bath/heating block
- Powder-free gloves
- Clean, blunt forceps
- Micropipettors
- Sterilized and nuclease-free pipet tips
- Sterilized and nuclease-free microcentrifuge tubes
- High-speed microcentrifuge
- Low-speed tabletop microcentrifuge with slide holder attachment
- Vortex mixer
- Hybridization oven
- Hybridization accessories: chamber cover slides, etc.
- Rectangular slide staining dish and slide rack for washing microarrays
- PCR (polymerase chain reaction) machine
- Microarray scanner for standard 1” x 3” format (see Table 8 under “OneArray Microarray Scanner Specifications” for a list of compatible scanners)
- Hybridization systems (optional)
- Automated hybridization station (optional)

Other Necessary Reagents (Not Supplied)

Reagents

- De-ionized nuclease-free **water**
- Cyanine 3- or 5-labeled amplified **aRNA** sample, **or** Cyanine 3- or 5-labeled **cDNA** sample
- **20X SSPE stock solution**, sterile filtered:
    - 3.6 M Sodium chloride
    - 0.2M Sodium phosphate (pH 7.7)
    - 20mM EDTA
- **Wash Solutions**, sterile filtered (four types, approximately 250 mL of each is required per experiment):
    - 2X SSPE, 0.1% SDS
    - 2X SSPE
    - 0.1X SSPE, 0.1% SDS
    - 0.1X SSPE
    - **NOTE:** SDS must be molecular biology grade.
- **Pre-hybridization Buffer**, prepared and sterile filtered immediately prior to pre-hybridization:
    - 5X SSPE, 0.1% SDS, 1% BSA
    - **NOTE:** BSA must be molecular biology grade.
- Deionized **formamide** to be added to the OneArray Hybridization Buffer prior to use (see Step 4A).
- RNA **Fragmentation Reagent and Stop Solution** (for hybridization using aRNA)
- **DNA** Blocking Mixture:
    - Ambion® sheared Salmon Sperm DNA (10 μg/μl), or
    - Invitrogen™ Cot-1 DNA® (2.5 10 μg/μl), or
    - Invitrogen™ Poly-A (2.5 10 μg/μl)

Important Notes on Microarray Handling and Storage

Storage Conditions

- Store unopened OneArray product at room temperature.
- Store opened OneArray product at 4°C.
- Store OneArray Hybridization Buffer at room temperature.

NOTE: If the product is received with an open bag, please contact Phalanx Biotech Customer Service for an immediate replacement.

Handling Microarrays

Please read this section carefully and follow the instructions!

- Polynucleotide probes are printed on the side of the slide with the barcode.
- To avoid irreparable damage of the printing area, **do not touch** the surface with bare hands, or with any other objects.
- Whenever possible, handle microarrays with clean blunt forceps to avoid contamination.

Open arrays should be used within a week.

Product Descriptions and Overview

OneArray™ Whole Genome DNA microarrays are made of sense-strand polynucleotide probes spotted onto a proprietary chemical layer coated on top of a 1” x 3” (25 mm x 75 mm) standard-format microarray glass slide. Updated information of genome content from public domains is used to design approximately 30,000 highly sensitive long-oligonucleotide probes for monitoring the expression level of corresponding protein-coding genes.

Each probe is spotted onto the array in a highly consistent manner using a proprietary, non-contact spotting technology adapted for microarray manufacturing.

Mouse OneArray Genome Content

Each microarray contains 31,802 oligonucleotides: *29,922 mouse genome probes*, and *1880 experimental control probes*.

Mouse OneArray content is based on an abridged version of the Mouse Exonic Evidence Based Oligonucleotide (MEEBO)*. MEEBO is a new set of 70-mer probes specifically for DNA microarrays that was created by a team of researchers from the University of California, San Francisco and elsewhere, and was headed by Dr. Ahs Alizadah. MEEBO has been made available to the public.

For more information about MEEBO, access the following Web sites:

http://arrays.ucsf.edu/archive/meebo.html

http://genome-www5.stanford.edu

* Special thanks to Dr. John Coller for his helpful discussion in the selection of probe sets for reduction.

Table 1, below, provides an example of the contents of a mouse genome that can be studied using the Mouse OneArray.

Table 1: Mouse Genome Content

Probe Type	Number of Probes
MEEBO (abridged version)	**29,922 (total)***
Group Code: MC **Mouse constitutive exonic: Rockefeller MouSDB3 Constitutive exons and locuslink2ucsf constitutive exons**	**24,858**
Group Code: MR **Mouse mRNAs; mRNA derived 70-mer probes, which may span >1 exon**	**5,064**

* Mouse OneArray is guaranteed to contain > 98% of the total probe content.

Mouse OneArray Control Features

There are 1,880 control probes built into the Mouse OneArray DNA microarray that monitor the sample quality and hybridization process. These control probes provide valuable information to ensure experiments are done correctly to ensure higher quality results for analysis.

SimpleMeasure™ is a small, free Java-based applet designed to analyze control probe data and generate easy-to-interpret graphs. The program can be downloaded from

http://www.phalanxbiotech.com/Support/Downloads.html

NOTE: Detailed control information, gene lists, gene annotations, and probe sequences can be found on the Product Support CD that accompanied this product, or at:

http://www.phalanxbiotech.com

Human OneArray Genome Content

Each microarray contains 32,050 oligonucleotides: *30,968 human genome probes*, and *1082 experimental control probes*.

Each oligonucleotide probe is designed to hybridize to a specific target gene described in the current public domain contents, such as UniGene, Cancer Genome Anatomy Project (CGAP), BioCarta, Kyoto Encyclopedia of Genes and Genomes (KEGG), and validated by the Human Genome Sequencing Project (HGSP).

Table 2, below, provides an example of the contents of a human genome that can be studied using the Human OneArray.

Table 2: Human Genome Content

Probe Type	Number of Probes
UniGene and RefSeq based	**30,968 (total)***
UniGene build #175 based and/or RefSeq based with Entrez Gene ID including: **CGAP (Cancer Genome Anatomy Project)** **BioCarts and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways**	**28,703**
UniGene build #163 based with Gene ID and experimentally selected	**2265**

* Human OneArray is guaranteed to contain > 98% of the total probe content.

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Human OneArray Control Features

There are 1,082 control probes built into the Human OneArray DNA microarray that monitor the sample quality and hybridization process. These control probes provide valuable information to ensure experiments are done correctly resulting in higher quality results for analysis.

SimpleMeasure™ is a small, free Java-based applet designed to analyze control probe data and generate easy-to-interpret graphs. The program can be downloaded from

http://www.phalanxbiotech.com/Support/Downloads.html

NOTE: Detailed control information, gene lists, gene annotations, and probe sequences can be found on the Product Support CD that accompanied this product, or at:

http://www.phalanxbiotech.com

Using OneArray

This section provides you with detailed information about how to perform the steps necessary to complete the hybridization process to study gene expressions using the OneArray microarray.

Follow these detailed steps *exactly* to achieve the best experimentation results.

- **Step 1:** Prepare the RNA Sample
- **Step 2:** Label the Target
- **Step 3:** Pre-Hybridize the Microarray
- **Step 4:** Perform the Hybridization Protocol
- **Step 5:** Wash the Hybridized Microarray
- **Step 6:** Scan and Extract Gene Expression Results
- **Step 7:** Check Control Probe Data

Step 1: Prepare the RNA Sample

High-quality, intact RNA is essential for all gene expression microarray experiments.

There are many different RNA isolation protocols and commercially available RNA isolation kits. You should choose a solution that meets your specific needs. Stratagene, Ambion, Invitrogen and other reagent companies offer many different RNA isolation products. For more information, you can visit each company's Web site.

Once the RNA samples are isolated, you must confirm the quantity and quality of the samples. Similarly, many different protocols are available and you should choose a solution that is suitable for your needs.

For faster and more automated RNA analysis, you may want to consider the "No Cuvettes" Spectrophotometer from NanoDrop®, or the 2100 Bioanalyzer from Agilent Technologies. For more information, visit each company's Web site.

Step 2: Label the Target

For best results, it is recommended that you use one of the commercially available labeling kits that has been tested for use with the OneArray microarray—please refer to Tables 3 and 4 below.

General Guidelines for Target Labeling

There are many commercially available labeling kits for microarray analysis. Select a labeling kit or labeling method that is most suitable for your specific needs. If you use a labeling kit that is not listed in Tables 3 nor 4, it is recommended that you validate the method to test and determine its compatibility with the OneArray.

You may want to confirm the quality of the labeled target with the "No Cuvettes" Spectrophotometer from NanoDrop®.

RNA Sample Amounts

Generally, the amount needed of quality RNA is 10-20 μg for each labeling reaction.

If you have an *ample* supply of RNA samples, you have the *choice* of using a protocol that either amplifies or does not amplify the RNA sample.

If you have a *limited* amount of RNA samples, it is recommended that you use a protocol that includes a linear amplification of the RNA samples.

Dye Incorporation Efficiency

Good dye incorporation rates are important for yielding the best data from microarray hybridization. Incorporation rates of 30-60 dye molecules per 1000 bases (17-33 bases/dye molecule) yield the most usable data. Rates below 20 dyes per 1000 bases (50 bases/dye) are very low and may lead to a loss of signal of many

targets. It is not recommended to perform hybridization with samples of low dye incorporation efficiency.

For aRNA Hybridization

Follow the instructions provided by the reagent supplier. Indirect labeling with NHS ester dye is recommended. Table 3, below, contains a list of products that have been tested for use with OneArray.

Table 3: aRNA Preparation Products

Manufacturer	Product Name and Description
Ambion®	Amino Allyl MessageAmp II™ aRNA Kit
Ambion®	aRNA Fragmentation Reagent
Epicentre® Biotechnologies	TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit

For aRNA labeling, >10 μg of quality aRNA is recommended. Smaller volumes can lead to significant loss of sample and may increase the concentration of contaminants in the labeled aRNA sample, leading to higher background signal.

It is best to use aRNA as soon as possible after labeling, as exposure to air and light can reduce the signal of some dyes. If it must be left overnight, it is best to aliquot your labeled aRNA and store in the dark at -80°C. Avoid thawing and re-freezing aRNA if possible, as freeze-thaw cycles can damage the aRNA.

Finally, aRNA fragmentation is best performed immediately prior to hybridization (Step 4B).

For cDNA Hybridization

Follow the instructions provided by the reagent supplier. Indirect labeling with NHS ester dye is recommended. Table 4, below, contains a list of products that have been tested for use with OneArray.

Table 4: cDNA Preparation Products

Manufacturer	Product Name and Description
Ambion®	**Amino Allyl MessageAmp II™ cDNA Kit**
Invitrogen™	**Superscript Indirect Labeling System**
Stratagene®	**Fairplay II Microarray Labeling Kit**

For cDNA labeling, it is recommended that 15 μg of total nucleic acid be used as starting material.

For all cDNA preparation, it is strongly recommended that you re-purify the targets using the Qiagen® Mini-Elute PCR purification column prior to hybridization according to the manufacturer's protocol. Incomplete removal of unincorporated dye from the labeling reaction often contributes to increased background noise. The Qiagen Mini Elute PCR has been found to consistently provide more reliable results.

Step 3: Pre-Hybridize the Microarray

General Instructions

OneArray requires a pre-hybridization step prior to hybridization of the labeled target. The pre-hybridization step reduces background signals and increases the performance of the microarray. Complete the pre-hybridization step by carefully following the instructions below.

1) Warm the pre-hybridization solution (5X SSPE, 0.1% SDS, and 1% BSA) to 42°C.
2) Pour 25 ml room temperature 100% ethanol into the spare array tube.
3) Preheat the OneArray(s) in the round cap tube at 60°C for 10 min (hybridization oven recommended).
4) Remove the OneArray(s) from the round cap tube, place in the two outermost slots inside the tube containing 100% ethanol, close the cap, and let sit for approximately 15 sec.
5) Shake the round cap tube for 20 sec.
6) Remove and thoroughly rinse each array with deionized water to remove any residual ethanol.
7) Carefully and slowly, fully submerge the OneArray in an abundant amount of pre-hybridization solution for 2 hr at 42°C (35 ml is sufficient if using a round cap tube).

Try to insert the slides into the correct position the first time. Avoid inserting and removing the slides more than once in the pre-hybridization buffer.

8) After 2 hr, transfer the slide(s) to room temperature, distilled water and wash gently for 2 min.
9) Spin dry the slide(s) for 2 min. Store in a dry, dark place until hybridization. It is recommended that you use the slides in the hybridization protocol within 1 hr of completing the pre-hybridization process.

Step 4: Complete the Hybridization Protocol

Once you have completed the pre-hybridization step using one of the methods outlined in the Step 3: Pre-Hybridize the Microarray section, you are ready to complete the hybridization protocol.

There are many different hybridization protocols, apparatus, and instruments available that may be compatible for use with the OneArray microarray. Detailed instructions for using the glass cover slide method are described below.

For best performance and consistent hybridization results, it is recommended that you use the OneArray Hybridization Buffer™, included with this product to complete the hybridization process.

Step 4A:➔Prepare Hybridization Solution Using the OneArray Hybridization Buffer (Included)

For correct use of this buffer, you must add a specific amount of formamide and labeled target. Please follow the instructions below carefully.

1) Spin down the stock OneArray Hybridization Buffer (~410 µl in each tube).
2) Add 90 µl of deionized formamide.

3) Warm the mixture to 42°C to completely dissolve the solution. Mix thoroughly.
   Yield: 500 µl of 1.5X Hybridization Buffer solution.
4) Aliquot the solution into individual tubes according to usage and store in darkness at -20°C.

Step 4B:➔Prepare Target for Hybridization

Hybridization Using Labeled Targets from aRNA *or* cDNA Labeling Approaches

For aRNA Hybridization

1) Mix 2 µg of your aRNA sample with nuclease-free H_2O to yield a final volume of 9 µL.

   NOTE: It is essential to use at least 2 µg of labeled target for each hybridization. If you are performing a dual-dye experiment, use at least 2 µg of *each* labeled aRNA sample.

2) Add 1 µl 10x Fragmentation Reagent, and incubate at 70°C for 15 minutes.
3) Add 1 µl Stop Solution, and mix well.
4) Mix with nuclease-free H_2O to yield a final volume of 17 µL.
5) Keep on ice and in darkness until hybridization (Step 4C).

For cDNA Hybridization

1) Mix 15 µg of your cDNA sample with nuclease-free H_2O to yield a final volume of 17 µL.

   NOTE: When using cDNA as your target, fragmentation is **not** necessary.

2) Keep on ice and in darkness until hybridization (Step 4C).

Step 4C:➔Complete the Hybridization Using the Glass Cover Slide Method

NOTE: If you perform hybridization using methods other than the basic glass cover slide method, it is recommended that you validate the protocol experimentally. For example, the MAUI System from BioMicro Systems, or HS Series of Hybridization Stations from TECAN offer a higher throughput and more automated hybridization methods.

To complete this step, you will need to select a type of glass cover slide. Table 5, below, contains a list of glass cover slides that have been tested and confirmed compatible for use with the OneArray Buffer.

Table 5: Compatible Glass Cover Slide Products

Manufacturer	Product Name
BioRad® Laboratories	**SLS 6001 (24x60 mm)**
Erie Scientific Company®	**mSeries LifterSlip™ 25x601-M-5439**
Corning®	**Cover Glass (24 X 60 mm)**

1) Ensure your work and experimentation area, as well as the OneArray, are clean before adding the Hybridization Buffer solution to the target array.
2) Pre-warm the Hybridization Buffer with formamide at 42°C for 10 minutes.
3) Prepare the hybridization mix in a 1.5 ml Eppendorf tube according to the Table 6, below.

Table 6: Hybridization Mix Measurements	
For each slide: 55 μl	
Component	***Final Volume***
1.5X OneArray Hybridization Buffer	**37 μl**
Sheared Salmon Sperm DNA (10 μg/μl)*	**1 μl**
Target preparation plus nuclease-free ddH_2O	**17 μl**

* Alternatives to Salmon Sperm DNA Blocking Mixtures:
Ambion® sheared Salmon Sperm DNA (10 μg/μl), or
Invitrogen™ Cot-1 DNA® (2.5 10 μg/μl), or
Invitrogen™ Poly-A (2.5 10 μg/μl)

4) Spin down the mixture for 5 minutes to eliminate potential debris.
5) Transfer the mixture to a new tube.
6) Heat the mixture to 95°C for 5 minutes (thermocycler recommended).
7) Maintain the mixture at a temperature of 60°C until pipetting onto the array (thermocycler recommended[1]).
8) Place the OneArray slide, bar code up, atop the "Probe Printed Region Guide" (included, see Figure 1).

[1] It may be helpful to set a Denature program in the thermocycler as follows:
95°C – 5 minutes
60°C – Hold

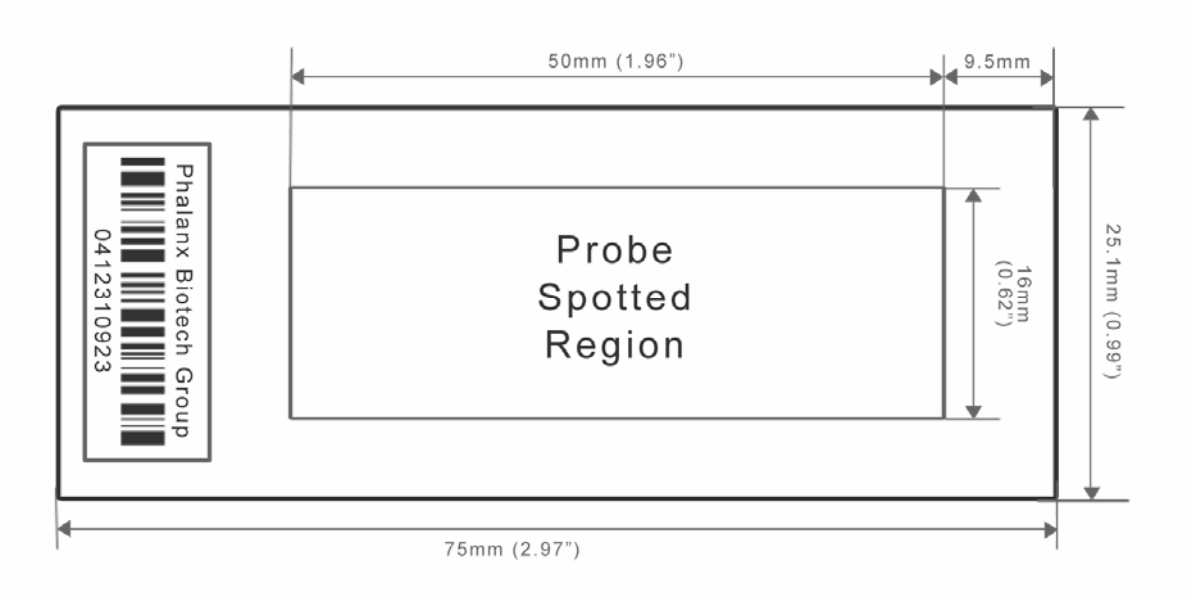

Figure 1: OneArray Microarray Glass Slide with "Probe Printed Region Guide" Plastic Underlay

9) Pipette the hybridization mixture onto the spotted region of OneArray DNA Microarray. Avoid creating any bubbles.

10) Carefully place the glass cover slide over the spotted area in an even manner.

11) Place the entire labeled target plus the microarray set-up into a closable, chambered box* that is humidified by 2X SSPE buffer in the 42°C oven for 14 to 16 hours. A sealed chamber ensures that the appropriate humidity level is maintained during incubation. (See Figure 2).

Figure 2, below, provides an illustration of Step 4C, where the hybridization protocol is completed using the glass cover slide method, and specifically, the OneArray DNA Microarray is placed into the chambered box.

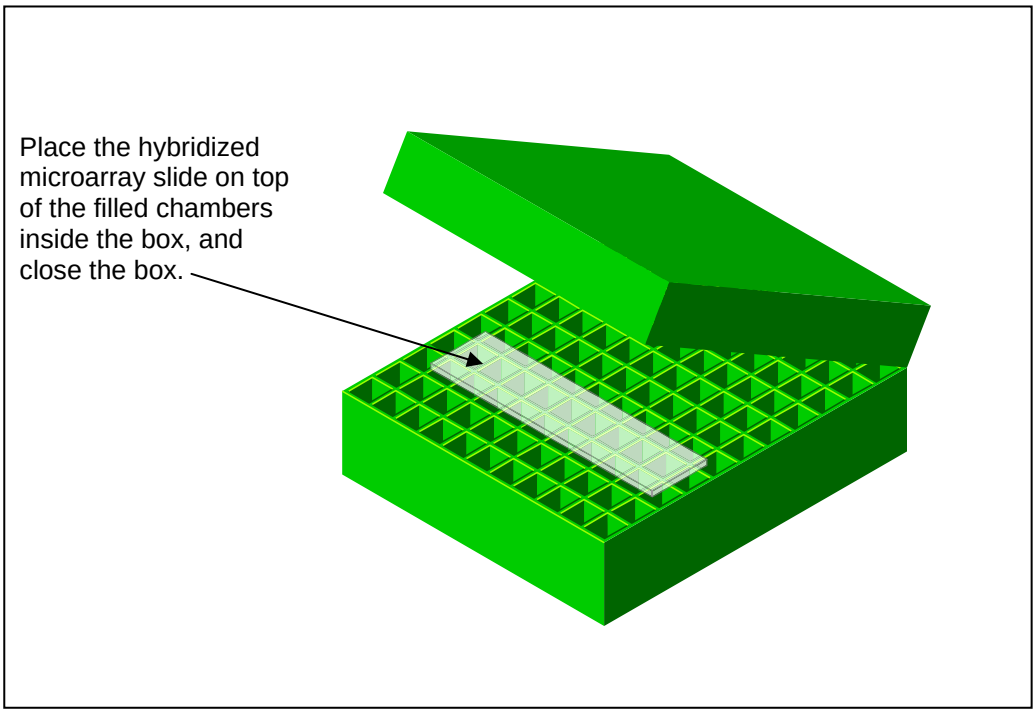

Figure 2: Step 4C → aRNA or cDNA Hybridization—Glass Slide Inside Chamber Box[2]

[2] The Hinged 100-Place Storage & Freezer Polypropylene Box from USA Scientific has been used to complete this step with frequent success. The small (approximately ½ inch x ½ inch) chambers within the box are filled about ¾ full of buffer, then the microarrays are laid on top of the chambers. The box is then closed and placed inside the oven. For information about this product or other USA Scientific products, access their Web site at: www.usascientific.com

Step 5: Wash the Hybridized Microarray

Washed and dried microarrays should be scanned within a couple of hours.

NOTE: Do not allow the microarray(s) to be exposed to air for a significant amount of time; otherwise, an increased fluorescent background signal could appear.

1) Submerge the entire labeled target and microarray set-up with the cover slide still intact into a large container filled with 42°C 2X SSPE, 0.1% SDS solution.
2) Carefully remove the cover slide from the glass by gently shaking the glass slide so that the cover slide is freed while the slide is submerged.

   NOTE: At this stage, the microarray has the highest concentration of unhybridized target and dye. Transfer the array quickly to the slide rack to minimize exposure to air.
3) Wash the slide(s) in the "rectangular, slide staining dish and slide rack" with the excess amount of pre-warmed 2X SSPE, 0.1% SDS solution for 5 min at 42°C.
4) Transfer the slide rack to a second slide staining dish that contains 0.1X SSPE, 0.1% SDS solution and wash for 5 min at room temperature.
5) Transfer the slide rack to a third slide staining dish that contains 0.1X SSPE and wash for 5 min at room temperature.
6) Rinse each array carefully with 0.1X SSPE using a squeeze bottle.
7) Spin dry with a centrifuge for at least one minute.
8) Keep the microarray dry and in the dark until ready to scan.

Step 6: Scan and Extract Gene Expression Results

There are many scanners available to extract signals from OneArray. Data extraction using GenePix™ 4100 from Molecular Devices is described below. Please refer to the respective company product instructions for appropriate use.

Table 7, below, lists the setting for using the GenePix 4100.

For a list of scanners that are compatible with the OneArray, please refer to Table 8, below.

NOTE: The performance of each scanner may differ. Therefore, to ensure best results, it is recommended that the scanner be adjusted based on standard microarray calibration parameters. Turn on and warm up the scanner for the duration according to manufacture instructions for the scanner.

Use the .gal file and Gene List provided with this product, or refer to our Web site at:

www.phalanxbiotech.com

Table 7: Scanner Settings Using GenePix™4100 from Molecular Devices

Wavelength	**635 nm**	**532 nm**
PMT	**630 V**	**590 V**
Minimum diameter (%)	**80**	
Maximum diameter (%)	**180**	
CPI Threshold	**100**	

NOTE: For lower versions of GenePix software, adjust the property parameter to 142.8 μm manually to obtain best results.

Figure 3, below provides a visual example of the OneArray glass slide with spotted probe region.

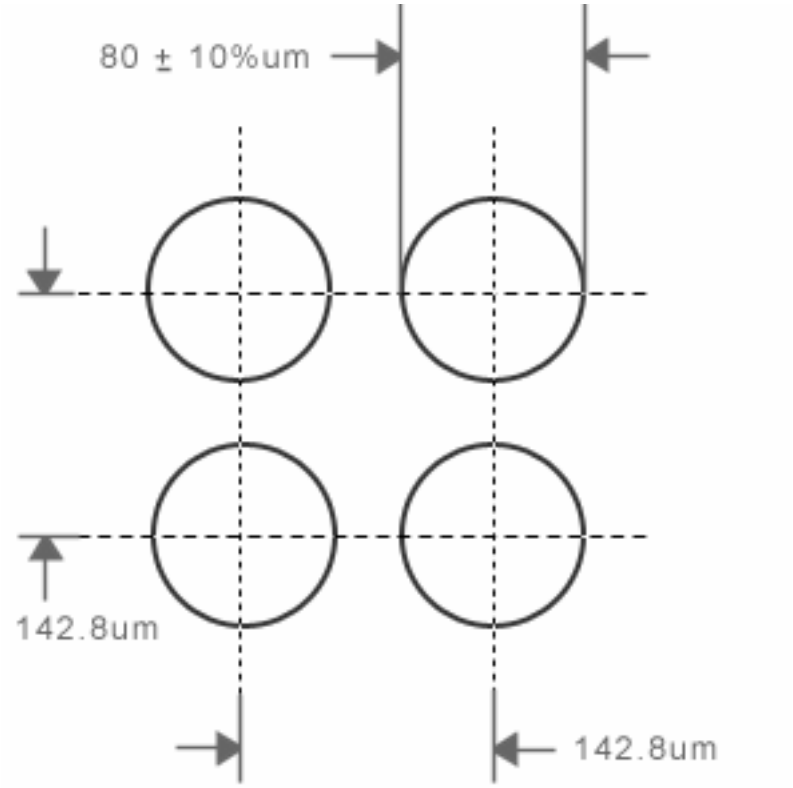

Figure 3: OneArray Glass Slide with Spotted Probe Region

OneArray Microarray Scanner Specifications

Select and use a microarray scanner that meets the specifications below.

Microarray Scanner Specifications

Format capabilities:	**1” x 3” (one inch by three inch) glass slide**
Molecular capabilities:	**Able to accurately detect, activate and read Cy3 and Cy5 fluorescent molecules**

Table 8, below, contains a partial list of microarray scanner products that are compatible for use with the OneArray microarray. Please refer to the respective company website for more information about the products listed below.

Table 8: Compatible Microarray Scanners

Manufacturer	Product Name and Description
Molecular Devices	Axon GenePix® 4000, 4100, and 4200 series
Genomic Solutions,® Inc.	GeneTAC™ 2000
Perkin Elmer,® Inc.	ScanArray™ 5000
TECAN®	LS 200/300/400
Agilent Technology	DNA Microarray Scanner G2565B

Step 7: Check the Control Probe Data

OneArray DNA Microarrays contains built-in control probes for performance monitoring of the hybridization process. They are used to confirm or deny whether the experiment was completed successfully. Please visit
http://www.phalanxbiotech.com/Support/Support.html

for more detailed information about the experimental controls on your OneArray product.

Alternatively, SimpleMeasure™ is a small, free Java-based applet designed to analyze control probe data and generate easy-to-interpret graphs. The program is included on your product CD or can be downloaded from

http://www.phalanxbiotech.com/Support/Downloads.html

Additional information about the control probes is included on the Product Support CD, and on our Web site at:

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